

Isolation of gbr22 protein

Introduction

Targeting proteins can be a difficult task, but by using certain methods, such as protein expression, purification, and characterization, isolating a specific protein is achievable. Proteins are usually synthesized in two steps, transcription and translation, inside prokaryotic and eukaryotic cells. With bacteria, specifically *Escherichia coli*, certain proteins can be expressed by introducing specific recombinant DNA to the cells. The recombinant DNA, pGEM-gbr22, will code for a fluorescent protein. Purple in color, the protein makes it easier to follow it in expression and purification. After *E. coli* cultures have grown into colonies while expressing the protein, the cells should be harvested by centrifugation, and stored away in a -20 C fridge. Protein purification is a series of processes used to isolate a single type of protein. Some of the ways to separate a protein is by exploiting differences such as protein size, binding affinity, and biological activity. The protein expressed from the bacteria has six histidine residues added to the C-terminus, which can be easily used to separate the protein from others. In a chromatographic procedure, histidine residues will bind to nickel with the presence of Ni-NTA agarose. To maximize purity, one should load the column with a slight excess over the predicted binding capacity. Once the protein is bounded to the Ni-NTA, it can be released by adding imidazole. Imidazole is an organic compound with the formula $C_3H_4N_2$. Once the protein is purified, the next step is to characterize it. Characterizing the purified protein is a method of monitoring the success of the previous steps. It is important to ensure that the protein is adequate quality and the correct one. Sodium dodecyl sulfate polyacrylamide gel electrophoresis is one of the techniques used to separate protein and other macromolecules. Electrophoresis refers to the movement of a charged particle in an electrical field. In gel electrophoresis, samples are introduced at one end of a porous gel and an electrical field is applied across the gel using a power supply. SDS is an anionic detergent that denatures protein and is used to analyze the distance of migration through the gel. The importance of the 3 protein methods is to obtain a yield of a single purified protein.

Materials and Methods

Protein Expression (1st Part)

The first steps to over expressing a bacteria is to insert a plasmid. About 1 ul of plasmid was gently added to the transformation tube containing bacteria. Heat shock allowed the plasmid to enter the bacteria. The bacteria was allowed to grow overnight in a plate with SOC media. The next day in the morning, a single colony of *E. coli* was chosen, and transferred into a culture tube containing ampicillin. The tube was placed in a shaking incubator. 0.625 ml of culture from the tube was then transferred into a flask, and placed into the shaking incubator. On day 3, a sample of culture was dispensed into a eppendorf tube. Bacteria was poured into a 50ml conical tube, and centrifuged. Purple pellets were formed and extra liquid was dispensed.

Protein Purification (2nd Part)

E. coli cells expressing the purple protein were lysed, and clarified by centrifugation. The protein was then purified using the Ni-NTA affinity chromatography, and stored in a fridge at 4 C. Several other samples were collected in between the lab for analyzing purposes in the next lab.

Protein Characterization (3rd Part)

A SDS-Page gel, and in its entire assembly was used to migrate several samples of protein, including the purified sample. After the migration of protein in the gel, the gel was rinsed off using an orbital shaker. Imperial protein stain was used to improve the appearance of the gel. After an hour, the protein stain was removed, and the gel was rinsed again. The next day, the gel was dried using a drying bed.

Results

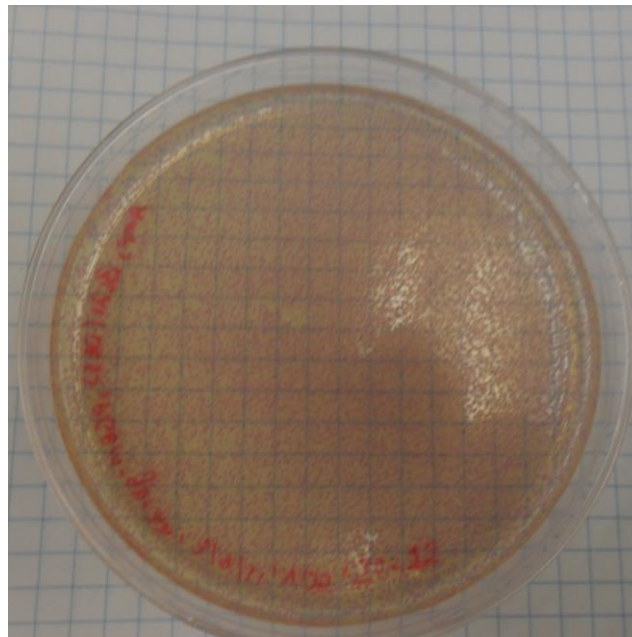


Plate containing ampicillin and pGEM-gbr22. 1 colony from this plate was selected for further experimentation.

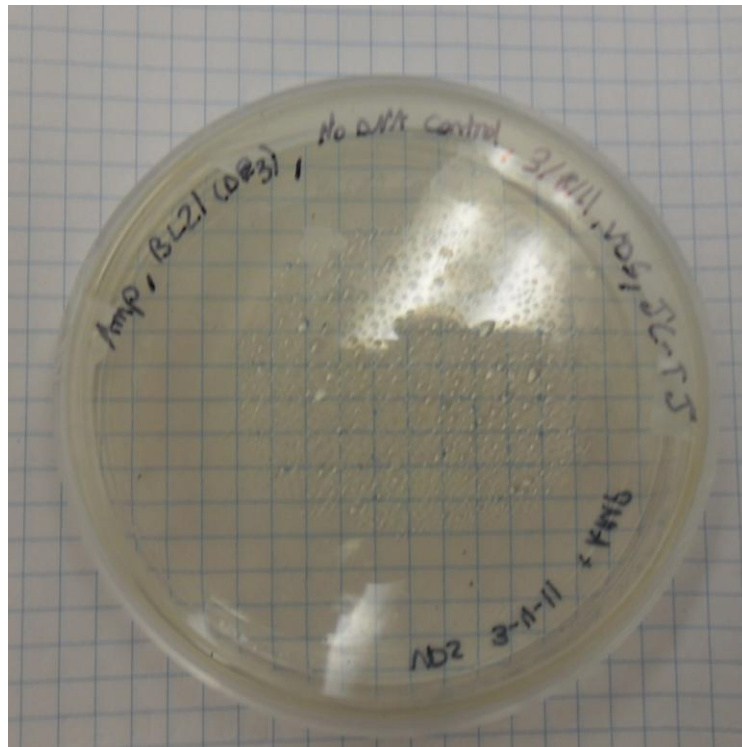
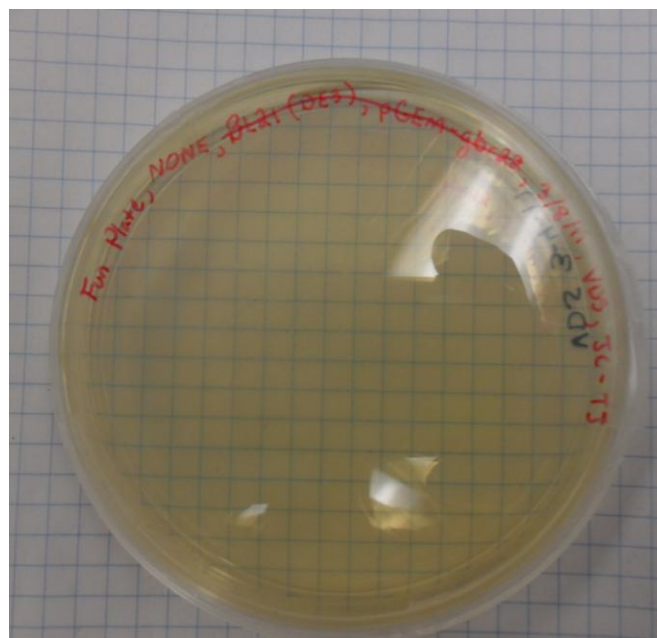
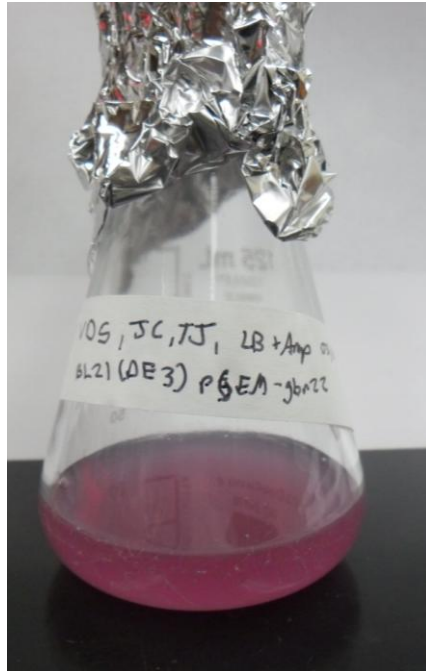


Plate containing no plasmid DNA. (Control Plate)



Fun Plate covered in partner's sneeze.



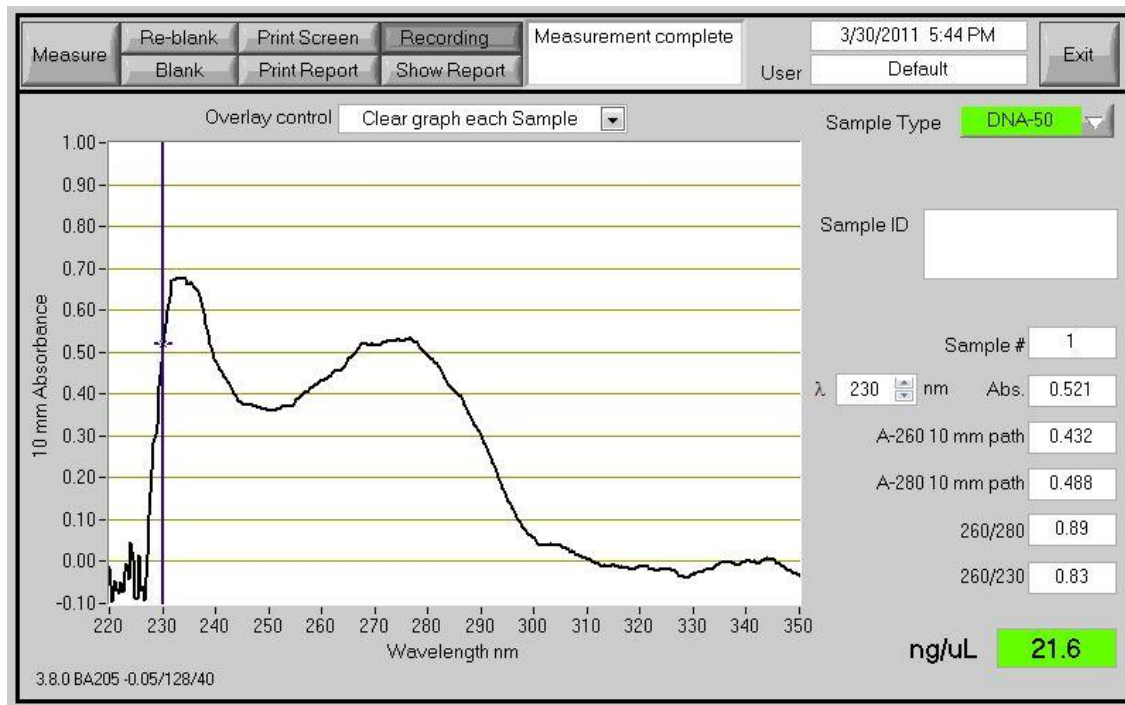
Flask containing rich purple culture. Purple indicated that the cells were ready to harvest.



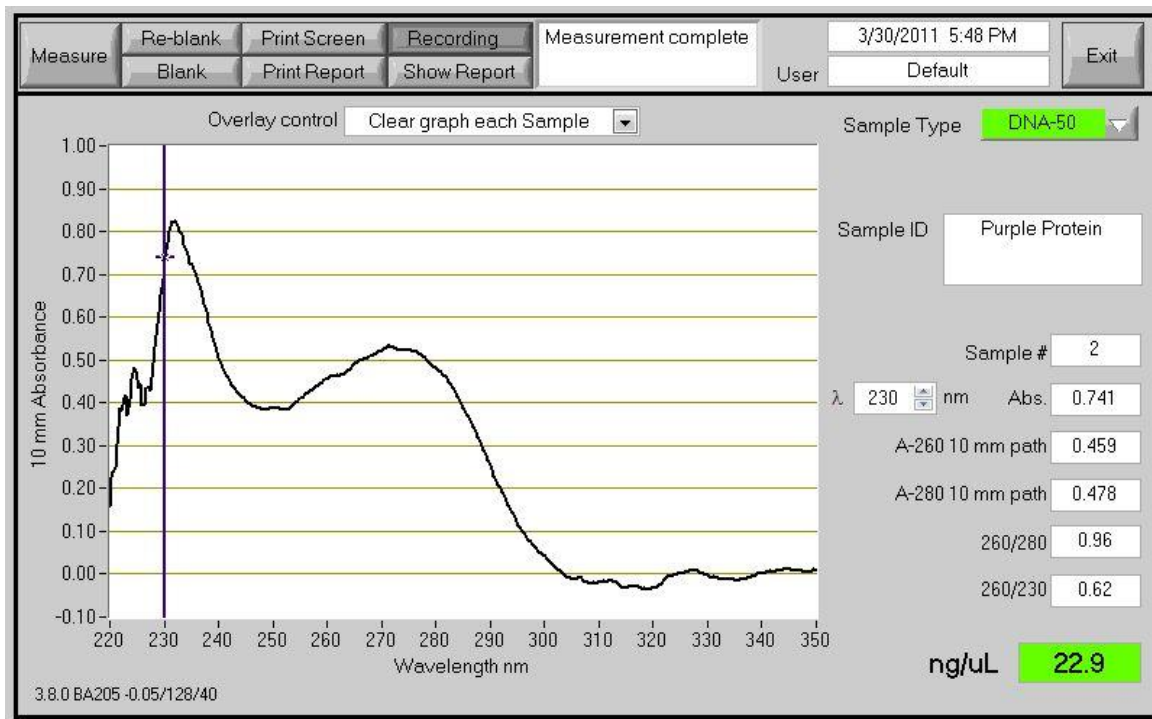
Cell pellet that formed after 10 minutes of centrifugation at 5000 rpm. The weight of the cell pellet was .37 grams.



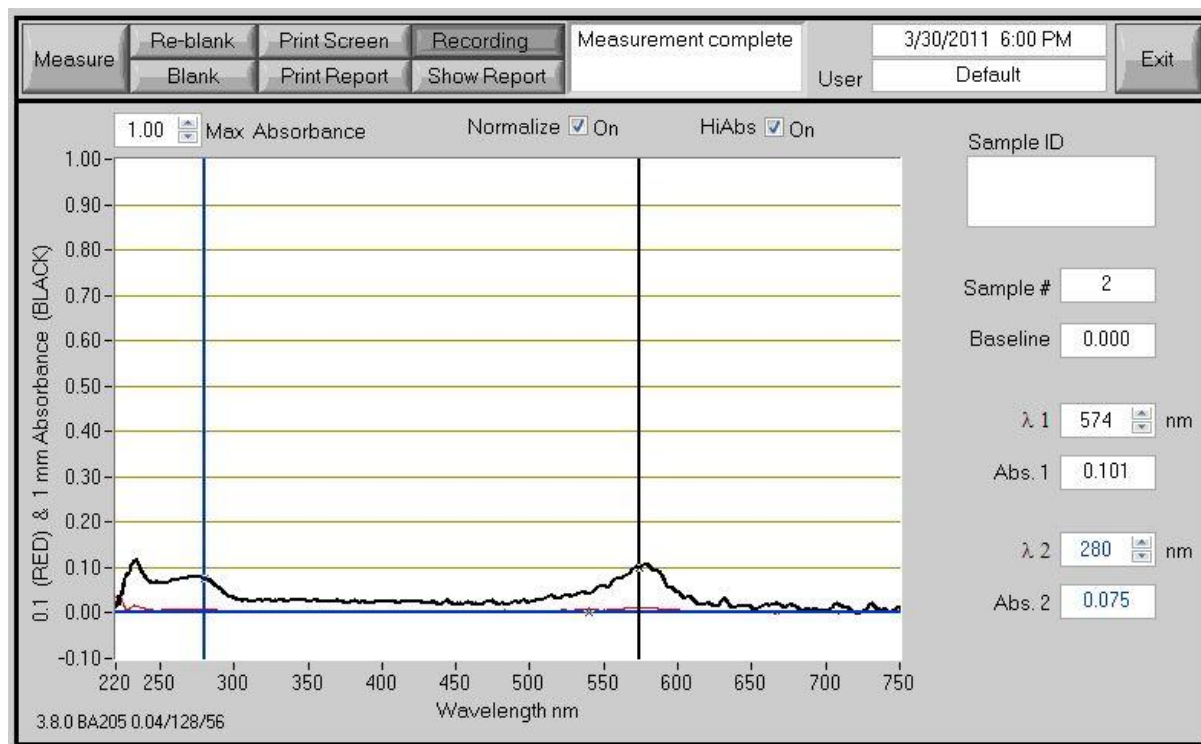
Elution 1 and Elution 2 in a 15 ml conical tube. Elution 1 was formed by adding 5 ml of the buffer containing 250 mM imidazole to the top of the column. Elution 2 was formed by adding 5 ml more.



First trial of Elution 1 using the Protein A-280 setting. Absorbance at 280 nm is 4.88.



Second trial of Elution 1 using the Protein A-280 setting. Absorbance at 280 nm is 4.78.



Comparison graph of the absorbance values at wavelengths 574 nm and 280 nm. Absorbances are 1.01 and 0.75. Absorbance values were multiplied by a value of ten, to get the path length to equal 1 cm. Absorbance has no units.

Beer's Law Calculations

$C = A/(Eb)$ Molecular Weight of gbr22 is 25794.2 g/mol

$\text{mols} = (M)(L)$

At 280 nm:

$$C = (0.75)/((39100 \text{ M}^{-1}\text{cm}^{-1})(1 \text{ cm}))$$

$$C = 1.98 \times 10^{-5} \text{ M}$$

$$\text{mols} = (1.98 \times 10^{-5} \text{ M})(50 \text{ ul or } .00005 \text{ L})$$

$$= 9.9 \times 10^{-10} \text{ mols} \times 25794.2 \text{ g/mol} = 2.55 \times 10^{-5} \text{ g or } 2.55 \times 10^{-2} \text{ mg of protein}$$

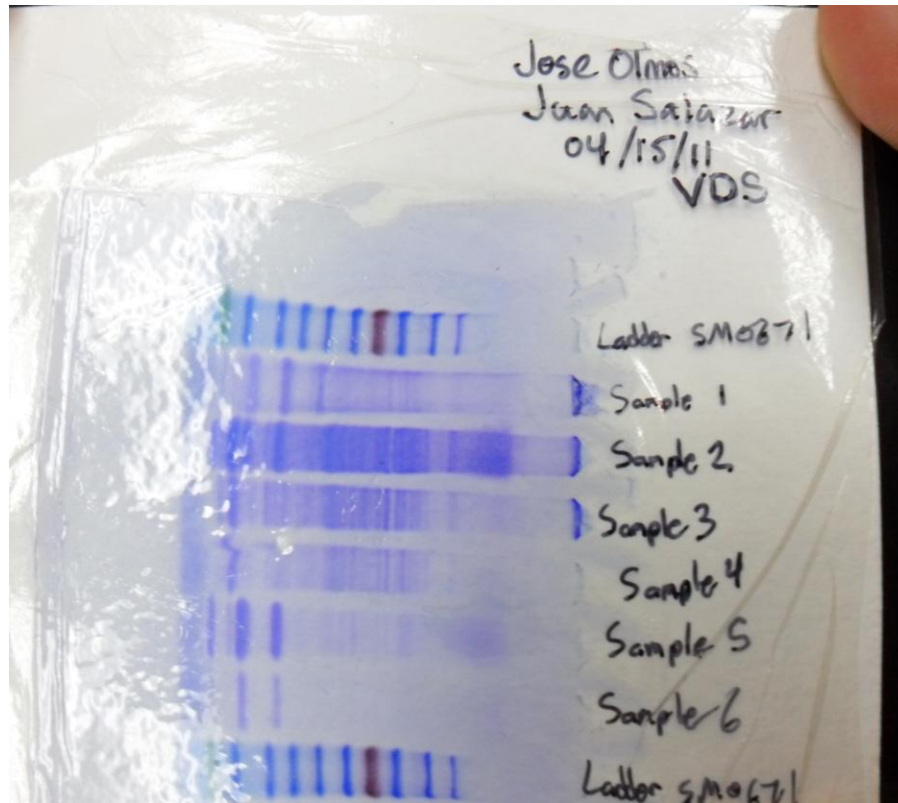
At 574 nm:

$$C = (1.01)/((118300 \text{ M}^{-1}\text{cm}^{-1})(1 \text{ cm}))$$

$$C = 8.54 \times 10^{-6} \text{ M}$$

$$\text{mols} = (8.54 \times 10^{-6} \text{ M})(50 \text{ ul or } .00005 \text{ L})$$

$$= 4.27 \times 10^{-10} \text{ mols} \times 25794.2 \text{ g/mol} = 1.10 \times 10^{-5} \text{ g or } 1.10 \times 10^{-2} \text{ mg of protein}$$



Lane 1 is blank. Lanes 2 and 9 contain Ladder. The ladder is called Ferments Page Ruler Prestained Protein Ladder #SM0671. Samples 1-6 are in lanes 3-8. There should only be one band visible in lane 8.

Discussion

Sample 6 was suppose to contain only one type of protein, or one band. Results showed 2 bands, which means something must of gone wrong in the experiment. It could have been that not enough Ni-NTA agarose was added into the column. Without enough Ni-NTA, the affinity for the intended protein will not be strong enough to efficiently purify. This lab also consisted of small and precise amounts of liquids to be dispensed using a micropipettor. If the micropipettor wasn't used correctly, the difference in amount would affect the results in the long run. The estimated purity would be about 50%. In sample 5, the estimated purity would be about 33%.

Conclusions

This lab taught several techniques that made it possible to isolate a specific protein. Although the results were not fully met, the overall procedure was done well. In order to improve the results, the entire experiment should be done again with extra caution. Dealing with proteins requires experience and lots of time. However, it is crucial that the correct protein is purified so it can be used in future labs. The protein yield obtained from this lab might be used in next VDS lab for testing out enzymes.

References

1. Protein production and purification. *Nature Methods* **2008**, 5 135-146.